

Steel DHW, Sandinha MT, White K. [The Plane of Vitreoretinal Separation and Results of Vitrectomy Surgery in Patients Given Ocriplasmin for Idiopathic Macular Hole](#). *Investigative Ophthalmology & Visual Science* 2015, 56(6), 4038-4044.

Copyright:

ARVO allows 'to publish the final version of the Article in the above Journal, and to distribute it and/or to communicate it to the public, either within the Journal, on its own, or with other related material throughout the world, in printed, electronic or any other form at or medium whether now known or hereafter devised.'

DOI link to article:

<http://dx.doi.org/10.1167/iovs.15-16409>

Date deposited:

26/04/2016



This work is licensed under a [Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International licence](#)

The Plane of Vitreoretinal Separation and Results of Vitrectomy Surgery in Patients Given Ocriplasmin for Idiopathic Macular Hole

David H. W. Steel,^{1,2} Maria T. Sandinha,¹ and Kathryn White³

¹Sunderland Eye Infirmary, Queen Alexandra Road, Sunderland, United Kingdom

²Institute of Genetic Medicine, Newcastle University, Newcastle Upon Tyne, United Kingdom

³EM Research Services, Newcastle University, Newcastle Upon Tyne, United Kingdom

Correspondence: David H. W. Steel, Sunderland Eye Infirmary, Queen Alexandra Road, Sunderland, UK; David.steel@ncl.ac.uk.

Submitted: January 9, 2015

Accepted: May 16, 2015

Citation: Steel DHW, Sandinha MT, White K. The plane of vitreoretinal separation and results of vitrectomy surgery in patients given ocriplasmin for idiopathic macular hole. *Invest Ophthalmol Vis Sci*. 2015;56:4038–4044. DOI:10.1167/iovs.15-16409

PURPOSE. We assessed whether ocriplasmin (OCP) given to patients with idiopathic macular hole (IMH) results in an altered plane of vitreoretinal (VR) separation compared to patients without OCP exposure.

METHODS. A prospective study was done of 12 patients with IMH and vitreomacular adhesion (VMA) given OCP. Patients in whom the IMH failed to close underwent vitrectomy with peeling of the internal limiting membrane (ILM). The intraoperative staining pattern of the ILM using Brilliant Blue G (BBG) and examination of the excised ILM with transmission electron microscopy (TEM) were compared to results of a matched cohort of 31 patients with IMH who had not received OCP.

RESULTS. Among 12 patients treated with OCP, VMA release occurred in 7 (58%) and hole closure was achieved in 3 (25%). Vitrectomy was done on the nine patients without hole closure. In seven of these nine (78%) the ILM had less than 5% of its vitreous surface covered in residual material on TEM, which was significantly less than in the control group (5/31, 16%, $P = 0.001$). In two OCP patients, large amounts of vitreous side material were present, but the rim of the hole stained evenly with BBG, suggesting that epiretinal material had avulsed with VR separation, a pattern not seen in any of the control patients. All patients had IMH closure after vitrectomy and visual results were not significantly different from the control group.

CONCLUSIONS. Ocriplasmin facilitates more complete VR separation in patients undergoing surgery for IMH, although it does not result in more eccentric epiretinal tissue release.

Keywords: ocriplasmin, Brilliant Blue G, Staining, internal limiting membrane, macular hole, vitrectomy, electron microscopy

Ocriplasmin (OCP) is a recombinantly made molecule containing the catalytic domain of the nonspecific serine protease Plasmin. It is thought to exert its action by catalysing the cleavage of peptide bonds on the carboxy-terminal side of lysine and arginine molecules in the adhesion molecules laminin and fibronectin that bond the cortical vitreous to the internal limiting membrane (ILM) of the retina.^{1,2} One of the proposed advantages of OCP, when given intravitreally to induce vitreoretinal (VR) separation, is that it results in a cleaner plane of separation compared to surgically induced or spontaneous vitreous detachment.^{3,4} This is based on preclinical animal and postmortem human studies in subjects with normal physiological VR adhesion.^{5,6} Ocriplasmin has been licensed for the treatment of symptomatic vitreomacular adhesion (VMA) with or without accompanying idiopathic macular hole (IMH). It is successful in inducing VR separation in approximately 40% of patients if there is no accompanying epiretinal membrane (ERM) and results in IMH closure, if present, in approximately the same proportion.² It is unknown if the VR cleavage plane is altered in cases of VMA and IMH given OCP. This would be of interest as it is well documented that residual epiretinal tissue commonly is present after

vitrectomy for IMH and its removal is one of the principal motivations for peeling the ILM.^{3,7,8} Furthermore, it is possible that OCP may alter the adhesion of the ILM to the underlying Müller cell end plates by its action on laminin and fibronectin deep to the ILM.

We performed a prospective observational study to ascertain the plane of VR separation in patients with IMH given OCP, but who failed to close and who then underwent vitrectomy surgery. Furthermore, we also evaluated the plane of ILM separation from the underlying retina and the results of surgery.

METHODS

Consecutive patients undergoing intravitreal injection with OCP (Jetrea; ThromboGenics, Leuven, Belgium) for idiopathic IMH of less than 400 μm in minimum linear diameter with VMA were included in the study. Patients in whom the hole failed to close at 1 month following injection were offered surgery with vitrectomy, ILM peeling, and gas. The study followed the tenets of the Declaration of Helsinki, with approval from the local institutional review board. Informed consent was obtained from the subjects after explanation of the nature of the study.

All patients underwent transconjunctival 25-gauge (25g) vitrectomy (Constellation; Alcon, Fort Worth, TX, USA) using wide-field noncontact viewing (Eibos; Haag-Streit, König, Switzerland) with combined phacoemulsification and intraocular lens (IOL) implantation if phakic. During surgery the presence of any vitreoretinal adhesion was assessed using diluted triamcinolone staining and, in cases where this was present, posterior hyaloid face separation was achieved with aspiration.

Brilliant Blue G (BBG, ILM Blue; DORC International, Zuidland, The Netherlands) was used to stain the macula in all cases. This is a highly purified preparation of 0.025% BBG mixed with 4% polyethylene glycol to produce a heavier than water solution. The dye was refluxed onto the macula retina using the vitrectomy probe and aspirated off after 5 seconds of contact time. A macular contact lens was used to view the peeling procedure. The ILM was peeled using a pinch technique and Grieshaber DSP 25g end gripping forceps (Alcon Grieshaber, Schaffhausen, Switzerland) and a peel radius of approximately 1 disc diameter. In cases where there was incomplete staining of the ILM with adherent pre-ILM tissue, an area of ILM that had normal staining was selected and the peel initiated from there. The pre-ILM tissue and ILM were, hence, peeled “en bloc,” without the need for a second peel. All surgeries were video recorded for later analysis of the dye staining pattern and characteristics as previously described.⁹

Either 25% SF6 or 20% C2F6 gas was used as a tamponade agent, and the patients instructed to position face down for at least 5 hours per day for 3 days. Patients were reviewed at 2 weeks and 3 months postoperatively. Pre- and postoperative best corrected visual acuity (BCVA) at 3 months following the last interventional procedure was measured using a standard Snellen acuity chart and converted to the logMAR scores for the purposes of statistical analysis.

Patients underwent spectral-domain optical coherence tomography (SD-OCT) on the Heidelberg Spectralis (Heidelberg Engineering, Heidelberg, Germany) immediately before and 1 and 4 weeks after OCP, and immediately before vitrectomy and 3 months postoperatively to assess closure. In repeat scans, the AutoRescan function was used to optimize scan concordance. The AutoRescan depends on active eye tracking provided through TruTrack. The eye tracking creates a detailed retinal map and corrects for eye movements, so that each follow-up OCT scan is registered and locked to the baseline scan. The minimum linear diameter (MLD) of the hole was measured as previously described using the Spectralis measuring tools.¹⁰ The presence or absence of vitreous attachment to the IMH rim was recorded at all time points and the presence of any epiretinal tissue noted.

Transmission Electron Microscopy (TEM)

The ILM from the patients was placed and fixed immediately in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer. The ILM was enrobed in low-melting point agarose (4%) to form a small block. After secondary fixation in 2% osmium tetroxide, the samples were dehydrated in graded acetone, embedded in epoxy resin, and polymerized at 60°C. Ultrathin sections (70 nm) were taken at two levels through the block, stained with uranyl acetate and lead citrate, and viewed on a Philips CM100 transmission electron microscope (Philips/FEI Corporation, Eindhoven, Holland).

The amount of cellular tissue on the vitreous and retinal sides of the ILM was quantified using images taken at $\times 7900$ magnification from 14 randomly sampled areas of the ILM. To quantify the amount of debris on each surface of the ILM, a grid of lines (line length 2 μm) was superimposed on each image. The number of intercepts between the grid line and retinal or

vitreous surface were counted. Another grid (line length 1 μm) then was superimposed on each image, and the number of intercepts between the grid lines and any retinal or vitreous side tissue were counted. The percentage of surface covered by cellular tissue was taken as the number of intercepts on tissue/(number of intercepts on the ILM surface $\times 2$) $\times 100$ as previously described⁹ (and see Supplementary Fig. S1).

A “clean” vitreous surface of the ILM was defined as having less than 5% of the surface covered by residual vitreous or cellular material (Fig. 1).

The results of the analysis were compared to a group of patients with IMH less than 400 μm in diameter who had not been given OCP, but who also had undergone IMH surgery using an identical technique and ILM sampling procedure. These eyes were recruited in the immediate 12-month period before the availability of OCP.

Statistical Analysis

Descriptive and statistical analyses was performed using IBM SPSS Statistics 21 (IBM, White Plains, NY, USA). Patients' demographic characteristics, pre- and postoperative variables, and IMH features are presented in terms of mean, SD, and range or percentage as appropriate.

Two-sample *t*-tests were used to compare continuous variables and associations between noncontinuous variables were analyzed using the χ^2 statistic using Fisher's exact probability. Statistical significance was considered with a *P* value of 0.05 or less.

RESULTS

The study period was from October 2013 to November 2014. During this time period 12 eyes of 12 patients were treated with 0.125 mg intravitreal OCP. Vitreomacular adhesion separation was achieved in 7 of the 12 eyes (58%). In three of these eyes, macular hole closure occurred (25% of total) and no further intervention was done. Macular hole closure did not occur in any eye without VMA release. Baseline features for all 12 eyes are given in Table 1. There were significant differences in the mean age, MLD, base diameter, and visual acuity outcome between those with primary closure with OCP and those without (Table 2). Holes that closed primarily with OCP had a smaller difference between MLD and base diameter than those that did not (mean difference 70 [SD 27] vs. 427 [SD 244], μm respectively, *P* = 0.002).

In the remaining nine eyes, four with and five without VMA separation, the hole remained open and vitrectomy surgery was performed. In two of the eyes without complete VMA separation the width of the VMA decreased slightly from 502 to 460 and 109 to 54 μm (patients 4 and 9, Table 1). In the other eyes without complete VMA release the extent of VMA was unchanged.

One patient experienced a macular involving rhegmatogenous retinal detachment five days after OCP injection and had surgery, consisting of vitrectomy, ILM peeling, laser to peripheral retinal breaks, and SF6 gas combined with phacoemulsification and IOL implant at 1 week after injection. The time period from OCP injection to surgery for the other 8 patients was a median of 6 weeks (range, 4–9 weeks). In four of the nine cases a greater than 100 μm increase in the MLD was noted on SD-OCT immediately before surgery with a proportionately greater increase in base diameter (mean increase in MLD = 133 μm [SD 106], mean increase in base diameter = 459 μm [SD 345], *P* = 0.007; Fig. 2). The change in macular hole size did not vary significantly between those with

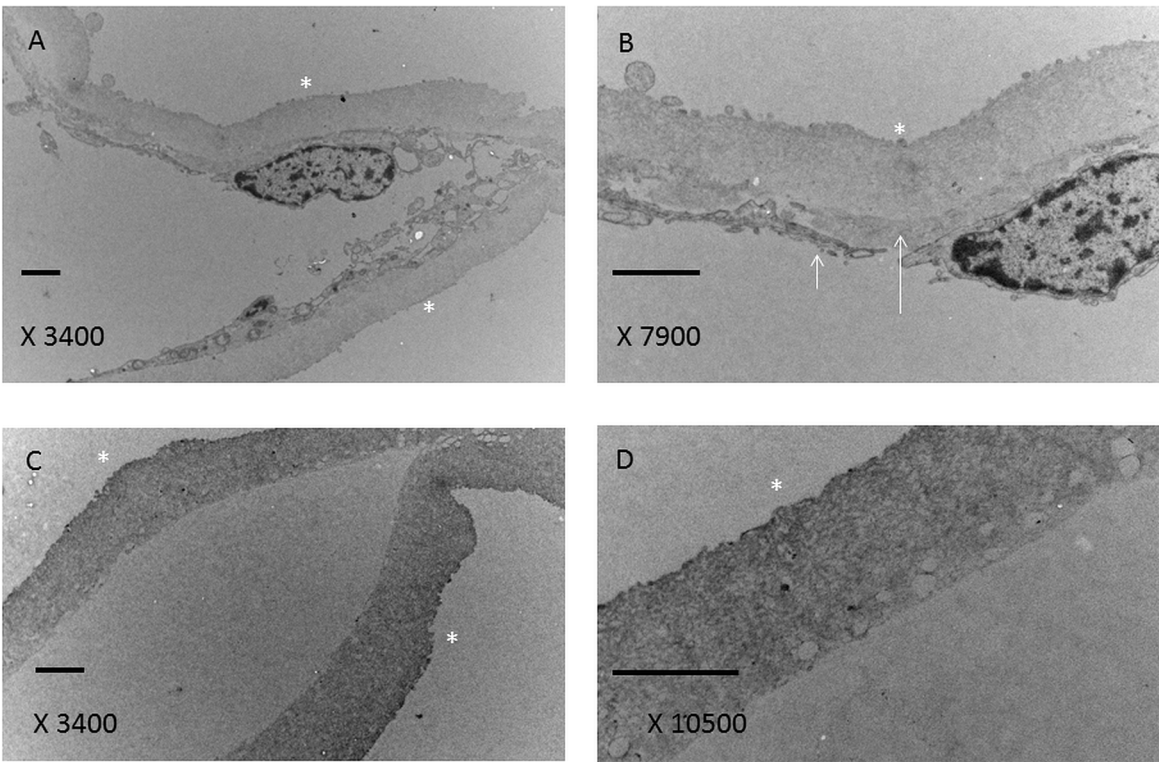


FIGURE 1. Representative TEM images of ILM. (A, B) Thin layer of adherent vitreous (*long arrow*) with an overlying cellular membrane (*short arrow*). (C, D) An ILM without any residual vitreous side material. *In all images the retinal side of the ILM is marked. *Scale bars:* 2 μ m.

and without vitreous separation ($P = 0.22$ for minimum linear diameter change and $P = 0.68$ for base diameter change).

The intraoperative staining pattern, and percentage of the vitreous and retinal sides of the ILM covered by abnormal tissue (residual cortical vitreous and cellular material on the vitreous side, Müller cell fragments on the retinal side) for the nine patients who underwent surgery for a persistently open hole are shown in Table 3. Seven of the nine patients had uniform staining patterns and only tiny fragments of residual vitreous/cellular material on the vitreous side of the ILM. None of the patients had a nonstaining rim around the holes; that is, none of the patients had epiretinal tissue surrounding the macular hole rim. Two of the nine patients had an unusual area of positive ILM staining surrounding the hole (i.e., the ILM

immediately surrounding the macular home rim did not have any epiretinal tissue overlying it) in the presence of large areas of nonstaining, representing ERM, more eccentrically (Fig. 3). Both patients had large amounts of vitreal side tissue on TEM, which appeared as a multilayered cellular membrane with a layer of vitreous between the cellular material and the ILM. Examination of the preoperative SD-OCT of these patients showed subtle ERM without vascular distortion on fundal photography, and this appearance was unchanged after OCP (Fig. 4). None of the other patients had evidence of any epiretinal tissue, either eccentrically or at the site of VMA on SD-OCT.

A control group of 31 eyes (31 patients) with IMH who had not received OCP, were used for comparative purposes to the 9

TABLE 1. Clinical Characteristics of All Eyes Given OCP

Number	Age	Sex	MLD/BD Before Injection, μ m	Vitreofoveal Separation After OCP	Closure After OCP	MLD/BD Before Surgery, μ m	BCVA 6 mo Postoperatively, logMAR
1	75	Female	312/757	Yes	No	644/1561	0.5
2	77	Male	281/687	Yes	No	526/1182	0.5
3	73	Male	222/380	Yes	No	*	0.3
4	75	Female	320/820	No	No	471/1607	0.5
5	68	Female	384/980	No	No	477/1432	0.8
6	73	Female	80/386	No	No	92/389	0.3
7	74	Male	240/412	Yes	No	302/660	0.2
8	79	Female	295/602	No	No	395/895	0.3
9	74	Female	371/1326	No	No	442/1912	0.6
10	66	Female	212/254	Yes	Yes	NA	0.2
11	68	Female	198/295	Yes	Yes	NA	0.2
12	69	Female	215/286	Yes	Yes	NA	0.3

NA, not applicable as hole closed; BD, base diameter.

* Retinal detachment involving fovea occurred 5 days following OCP and, hence, hole dimensions immediately before surgery uncertain.

TABLE 2. Comparison of Cases With and Without Primary Closure Following OCP

	Eyes With Macular Hole Closure Following OCP, <i>n</i> = 3	Eyes Without Macular Hole Closure After OCP, <i>n</i> = 9	<i>P</i>
Age, y, mean (SD)	68 (1.5)	74 (3)	0.001
Sex	All (100%) female	7 (78%) female, 2 (22%) male	1
Lens status	All (100%) phakic	7 (78%) phakic, 2 (22%) pseudophakic	1
Width of VMA, μ m, mean (SD)	303 (8)	180 (191)	0.09
MLD, μ m, mean (SD)	208 (9)	278 (91)	0.05
Base diameter, μ m, mean (SD)	278 (21)	540 (271)	0.02
Final visual outcome, logMAR, mean (SD)	0.23 (0.06)	0.44 (0.19)	0.01

eyes that failed to close after OCP (Table 4). The mean age of this group was 73 years ($P = 0.18$) and there were nine males ($P = 0.68$). Of the 31 eyes, 10 (32%) had VMA at the time of vitrectomy compared to 5 of the 9 (55%) in the OCP group ($P = 0.25$). The mean duration of symptoms before vitrectomy surgery was 9 weeks in the control group compared to 11 in the OCP treated group ($P = 0.52$). There also was no significant difference between the two groups for the MLD at baseline. Of the 31 eyes, 21 (67%) in the control group had a nonstaining rim compared to 0 of the 9 in the OCP group ($P = 0.0003$).

There were seven (78%) patients in the OCP group with a “clean” vitreous side of ILM surface compared to five (16%) in the control group ($P = 0.001$).

The BCVA at 3 months postoperatively in the OCP treated eyes was logMAR 0.44 (SD 0.19) and logMAR 0.35 (SD 0.19) in the control group ($P = 0.21$).

DISCUSSION

We found, as with preclinical studies on postmortem human eyes with a normal VR interface given OCP^{5,6} that the VR cleavage plane appears to be altered by OCP in cases where it has failed to result in IMH closure. The proportion of eyes with

a “clean” ILM after surgery was greater in OCP-treated eyes than our control eyes. This was the case in eyes with successful OCP-induced VR separation and those without. This concurs with the findings of Asami et al.,¹¹ who found that autologous Plasmin given preoperatively resulted in an improved cleavage plane in eyes with an attached, but otherwise normal vitreoretinal interface, regardless of whether vitreous separation was achieved pre- or intraoperatively. We also found significantly that none of the OCP-treated eyes had a non-staining rim (which would indicate epiretinal tissue) around the IMH, which we have described previously in approximately 50% of IMH cases.⁹

There were two eyes where significant amounts of vitreous and cellular remnants, representing an ERM, were left on the retina despite OCP. These also had the unusual finding of a rim of normally staining ILM around the hole, signifying no or limited vitreous or cellular material in this area despite the widespread more eccentric epiretinal material, which we have not seen in any other eyes. Interestingly both eyes had patchy high signal on the inner retinal surface on the preoperative SD-OCTs, which was not present on the other cases. Although there was no bridging traction or retinal distortion, the OCT appearance most likely represented the epiretinal tissue seen surgically and histologically. We hypothesize that when the

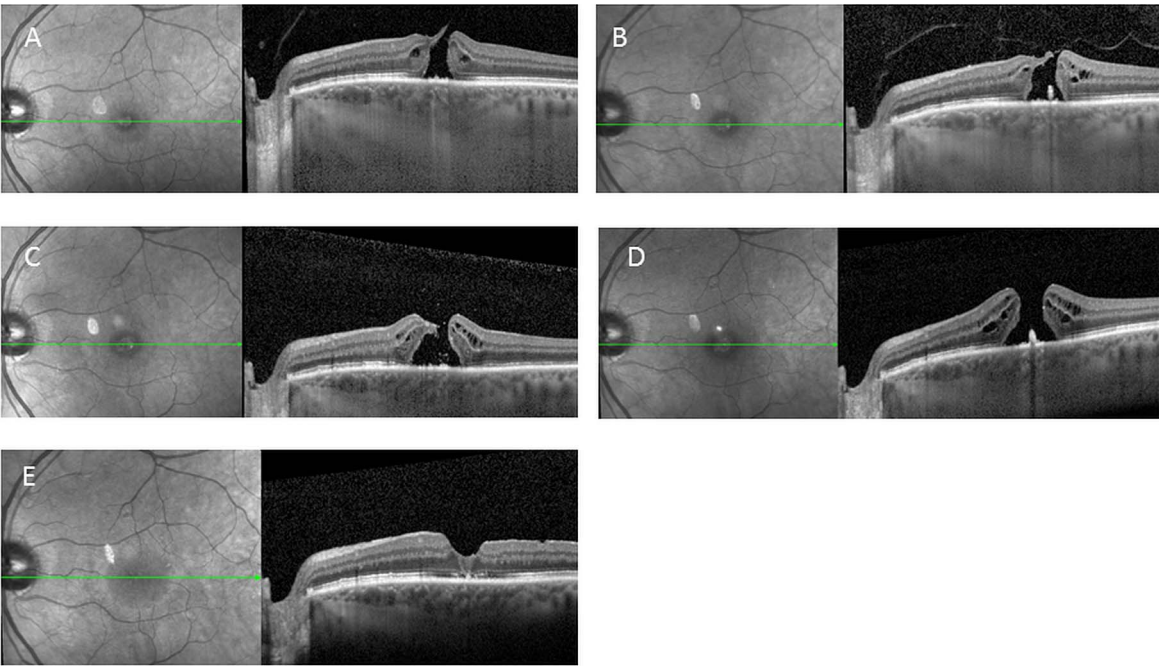


FIGURE 2. Horizontal line scan SD-OCT images of case number two at baseline before OCP (A), 1 week after OCP (B), 1 month after OCP (C), immediately before vitrectomy (6 weeks after OCP) (D), and 3 months after vitrectomy (E). Note release of VMA between 1 week and 1 month following OCP with increase in horizontal hole diameter, particularly base diameter.

TABLE 3. Intraoperative Staining Pattern and TEM Findings of Eyes Given OCP

Patient Number	% of ILM With Residual Tissue Observed		ERM Visible Preoperatively on OCT	Staining Pattern of BBG	
	Vitreous Side	Retinal Side		Across Macula	Immediately Around IMH Rim
1	2	23	—	Uniform pos.	Pos.
2	1	28	—	Uniform pos.	Pos.
3	0	20	—	Uniform pos.	Pos.
4	4	28	—	Uniform pos.	Pos.
5	72	49	+	Nonstaining	Pos.
6	78	28	+	Nonstaining	Pos.
7	2	18	—	Uniform pos.	Pos.
8	0	27	—	Uniform pos.	Pos.
9	0	19	—	Uniform pos.	Pos.

zone of vitreomacular adhesion in these eyes was forcibly separated at the time of surgery, the rim of cellular material around the IMH also separated. This is likely to be related to weakening of the adhesion between the remaining vitreous underlying the ERM and ILM by OCP. The more eccentric ERM remained attached to the retina, relating to the lack of attached vitreous to provide a tractional separation force, and also its action as a barrier to diffusion to the OCP. Despite the smaller molecular weight of OCP compared to plasmin, ERM will limit its diffusion to the VR interface, reducing its effectiveness. It also is known that some ERMs do not have an intervening layer of vitreous collagen between them and the ILM, although we did not find this in our cases.¹²

It is thought that most macular holes have a phase where vitreous is attached to the central foveal area with surrounding VR separation.¹³ In some cases when the vitreous separates spontaneously the developing IMH closes, although this is a rare phenomenon clinically.¹⁴ It is interesting to note that in the Microplasmin for Intravitreal Injection (MIVI) trials, OCP resulted in a greater hole closure rate than saline in cases where there was VR release. Ocriplasmin-induced VR separation from the fovea resulted in IMH closure in 43% of eyes in which VR separation was induced compared to 25% in the saline control arm of the MIVI trials.² Furthermore, even in cases where there was no VMA release, hole closure still occurred in 37% of cases compared to 6% in the saline arm (personal communication, data on file; ThromboGenics) This

would suggest that there is a further mechanism to hole closure other than VR separation alone. One possibility is that vitreous liquefaction with OCP reduces the tractional forces on the retina.¹⁵ Our findings suggesting a modified plane of VR separation compared to spontaneous or surgical separation around macular holes also may explain this. Even without total vitreous separation, local VR release may allow hole closure.

Conversely, the fact that four holes did not close despite complete VR separation and a clean ILM suggests that other factors must be addressed to achieve hole closure. The ILM contributes very significantly to the rigidity of the retina and its removal is known to improve macular hole closure.¹⁶ Changes in retinal morphology also are known to occur with advancing macular hole formation, such as those recently described by Woon et al.¹⁷ and ascribed to the central fovea having a bistable structure. We found that the three holes that closed primarily with OCP were smaller, and the mean age of the patients younger, than those that did not, both of which have been identified previously as being positive prognostic factors.² Also interestingly, the holes had relatively narrower base diameters compared to their MLD and this may be a positive prognostic factor, but larger numbers are needed to clarify this. We also observed an increase in diameter in all the holes that did not close after OCP, particularly base diameter. This could relate to vitreous traction, although we did not find any difference in the change in size between those with and without vitreous separation. Alternatively the increase in base diameter could

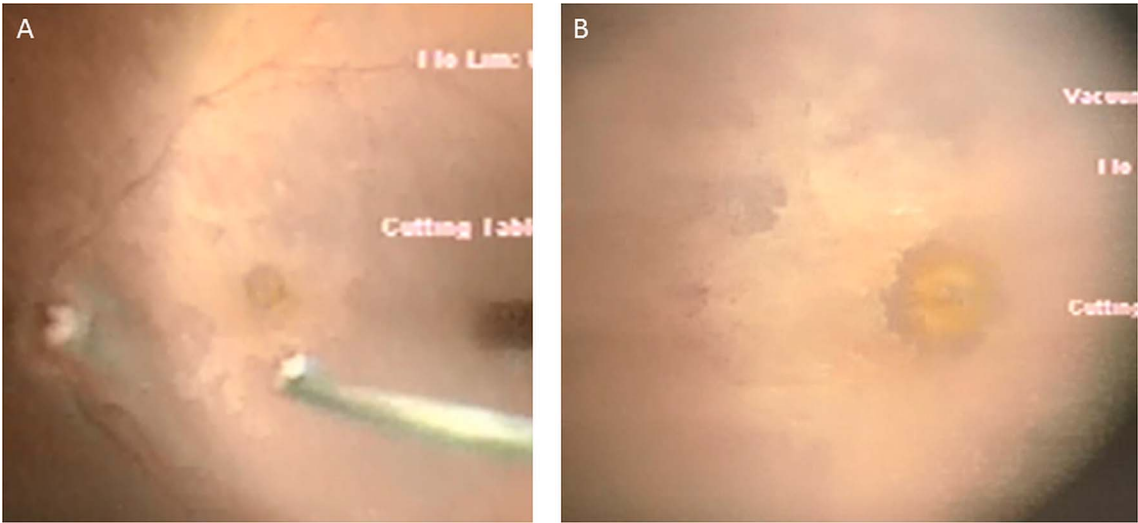


FIGURE 3. Intraoperative images of case number six, (A) with wide angle lens and (B) with high magnification contact lens in place, showing ILM after staining using BBG. Note area of positive staining around the macular hole rim with large areas on nonstaining, indicating epiretinal tissue eccentrically.

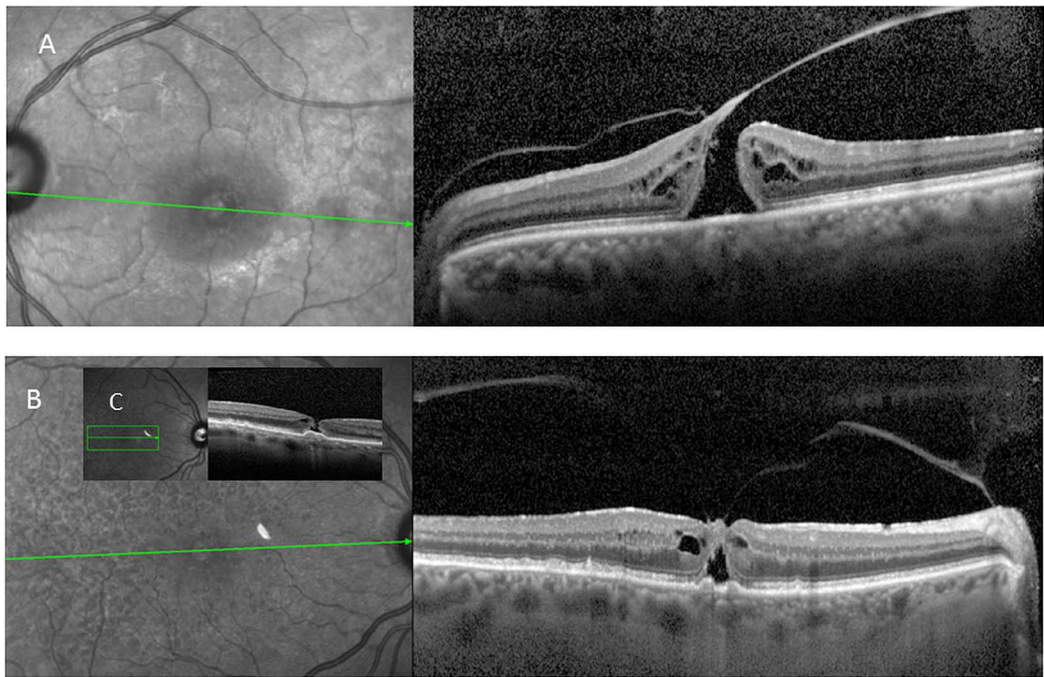


FIGURE 4. Horizontal line scan SD-OCT images of cases five (A) and six (B). Case six has a full-thickness retinal hole on eccentric slice (C). Note patchy high signal on retinal surface in both cases representing fine ERM.

relate to a direct outer retinal effect of OCP, which also could hinder hole closure despite successful VMA release.¹⁸ Recently, others have noted similar effects on macular hole size and the occurrence of new subretinal fluid after vitrectomy surgery with previous OCP use.^{19,20} We had no cases of nonclosure or subretinal fluid after vitrectomy surgery. We had one case of macular off retinal detachment after OCP due to the formation of three mid peripheral retinal breaks after vitreous separation. This case underwent successful retinal reattachment surgery combined with ILM peeling and a good visual outcome. Despite the increase in macular hole size and retinal detachment case we found no significant difference in visual acuity outcomes between our OCP-treated eyes and control eyes, although numbers are too small to draw any definitive conclusions regarding the effect of OCP on long-term visual outcome.

We did not find any changes in either the ILM itself or the cleavage plane of the ILM from its underlying layer of Müller cell endplates. The ILM is composed chiefly of type 4 collagen. Although referenced, but unpublished data, suggests that OCP may have some activity against type 4 collagen, others have not found this with Plasmin, and early phase studies with OCP have shown no changes in human post mortem and feline ILM.^{21,22} The exact adhesion mechanisms of ILM to Müller cells is uncertain and the lack of any change in ILM adhesion could have a number of explanations. The adhesion molecules responsible for ILM are incompletely documented, and the laminin subtypes and fibronectin isoforms may differ, and have different susceptibilities to those responsible for VR adhesion.

It also is possible that even if disrupted by OCP the adhesion molecules could have reformed by the time of vitrectomy surgery, which was a median of 6 weeks later. It also is possible that OCP did not diffuse to the sub-ILM space. We observed subtle ellipsoid changes around the macular hole margin in some of the patients, but no patient experienced dyschromatopsia or symptoms suggestive of more widespread photoreceptor dysfunction, as has been described previously, although we did not perform electrophysiology.^{23–26}

The study has several weaknesses. Larger numbers would add validity to our findings. We used TEM to quantify the extent of ILM surface material. Studies using scanning electron microscopy and flat-mounted specimens may be better at showing the exact arrangement of the residual vitreous material. However, we also documented the staining pattern of ILM-specific dye BBG, which we have shown previously correlates closely with the extent of pre-ILM material, and which added relevant topographical detail to our findings. As controls we used a series of ILMs from patients with macular holes of less than 400 μm in size; 32% of these had VMA at baseline compared to all nine OCP-treated eyes that underwent vitrectomy. However, 50% of the OCP-treated eyes released VMT following OCP, meaning that the two groups were more similar in this respect immediately before surgery.

In conclusion, this study suggested that OCP can lead to more complete VR separation in patients with macular holes and VMA. This was the case in eyes with OCP-induced VR separation and in eyes where the vitreous still needed to be

TABLE 4. Comparison of Eyes Given OCP to Eyes in the Non-OCP-Treated Control Group

	Age, y, Mean (SD, Range)	MLD at Baseline, μm , Mean (SD, Range)	% Tissue, Mean (SD, Range)		“Clean” Vitreous Side of the ILM, n (%)
			Retinal Side	Vitreous Side	
Eyes given OCP, n = 9	74 (3, 68–79)	264 (85, 80–384)	27 (9, 18–49)	18 (31, 0–78)	7 (78%)
Control eyes, n = 31	73 (6, 61–84)	288 (74, 148–399)	25 (8, 13–43)	29 (28, 0–78)	5 (16%)
P	0.14	0.33	0.58	0.35	0.001

separated at the time of surgery. Persistent VMA after OCP can occur in cases with ERMs surrounding the zone of VMA, which can be subtle, but discernible on preoperative SD-OCT. A clean ILM surface after OCP, however, does not guarantee macular hole closure and other morphological variants in macular hole shape and ILM rigidity are likely important.

Acknowledgments

Supported in part by an open grant from Alcon. The authors alone are responsible for the content and writing of the paper.

Disclosure: **D.H.W. Steel**, Alcon (F, C, R); **M.T. Sandinha**, None; **K. White**, None

References

- de Smet MD, Valmaggia C, Zarranz-Ventura J, Willekens B. Microplasmin: ex vivo characterization of its activity in porcine vitreous. *Invest Ophthalmol Vis Sci*. 2009;50:814-819.
- Stalmans P, Benz MS, Gandorfer A, et al. MIVI-TRUST Study Group. Enzymatic vitreolysis with ocriplasmin for vitreomacular traction and macular holes. *N Engl J Med*. 2012;367:606-615.
- Kishi S, Demaria C, Shimizu K. Vitreous cortex remnants at the fovea after spontaneous vitreous detachment. *Int Ophthalmol*. 1986;9:253-260.
- Gandorfer A. Objective of pharmacologic vitreolysis. *Dev Ophthalmol*. 2009;44:1-6.
- Gandorfer A, Rohleder M, Sethi C, et al. Posterior vitreous detachment induced by microplasmin. *Invest Ophthalmol Vis Sci*. 2004;45:641-647.
- Gandorfer A, Ulbig M, Kampik A. Plasmin-assisted vitrectomy eliminates cortical vitreous remnants. *Eye (Lond)*. 2002;16:95-97.
- Gandorfer A, Scheler R, Haritoglou C, et al. Pathology of the macular hole rim in flat-mounted internal limiting membrane specimens. *Retina*. 2009;29:1097-1105.
- Schumann RG, Schaumberger MM, Rohleder M et al. Ultrastructure of the vitreomacular interface in full-thickness idiopathic macular holes: a consecutive analysis of 100 cases. *Am J Ophthalmol*. 2006;141:1112-1119.
- Steel DH, Dinah C, Magdi H, White K, Rees J. The staining pattern of brilliant blue G during macular hole surgery: a clinicopathologic study. *Invest Ophthalmol Vis Sci*. 2014;55:5924-5931.
- DeCroos FC, Toth CA, Folgar FA, et al. Characterization vitreoretinal interface disorders using OCT in the interventional phase 3 trials of ocriplasmin. *Invest Ophthalmol Vis Sci*. 2012;53:6504-6511.
- Asami T, Terasaki H, Kachi S, et al. Ultrastructure of internal limiting membrane removed during plasmin-assisted vitrectomy from eyes with diabetic macular edema. *Ophthalmology*. 2004;111:231-237.
- Kampik A. Pathology of epiretinal membrane, idiopathic macular hole, and vitreomacular traction syndrome. *Retina*. 2012;32(suppl 2):S194-S198.
- Takahashi A, Yoshida A, Nagaoka T, et al. Idiopathic full-thickness macular holes and the vitreomacular interface: a high-resolution spectral-domain optical coherence tomography study. *Am J Ophthalmol*. 2012;154:881-892.
- Chew EY, Sperduto RD, Hiller R, et al. Clinical course of macular holes. The Eye Disease Case-Control Study. *Arch Ophthalmol*. 1999;117:242-246.
- Sebag J, Ansari RR, Suh KI. Pharmacologic vitreolysis with microplasmin increases vitreous diffusion coefficients. *Graefes Arch Clin Exp Ophthalmol*. 2007;45:576-580.
- Wollensak G, Spoerl E, Grosse G, Wirbelauer C. Biomechanical significance of the human internal limiting lamina. *Retina*. 2006;26:965-968.
- Woon WH, Greig D, Savage MD, et al. Movement of the inner retina complex during the development of primary full-thickness macular holes: implications for hypotheses of pathogenesis [published online ahead of print February 13, 2015]. *Graefes Arch Clin Exp Ophthalmol*.
- Chen W, Mo W, Sun K, et al. Microplasmin degrades fibronectin and laminin at vitreoretinal interface and outer retina during enzymatic vitrectomy. *Curr Eye Res*. 2009;34:1057-1064.
- Miller JB, Kim LA, Wu DM, Vavvas DG, Elliott D, Hussin D. Ocriplasmin for treatment of stage 2 macular holes: early clinical results. *Ophthalmic Surg Lasers Imaging Retina*. 2014;45:293-297.
- Hager A, Seibel I, Riechardt A, Rehak M, Jousen AM. Does ocriplasmin affect the RPE-photoreceptor adhesion in macular holes? *Br J Ophthalmol*. 2015;99:635-638.
- Stalmans P, Girach A. Vitreous levels of active ocriplasmin following intravitreal injection: results of an ascending exposure trial. *Invest Ophthalmol Vis Sci*. 2013;54:6620-6627.
- Liotta LA, Goldfarb RH, Brundage R, et al. Effect of plasminogen activator (urokinase), plasmin, and thrombin on glycoprotein and collagenous components of basement membrane. *Cancer Res*. 1981;41:4629-4636.
- Freund KB, Shah SA, Shah VP. Correlation of transient vision loss with outer retinal disruption following intravitreal Ocriplasmin. *Eye (Lond)*. 2013;27:773-774.
- Tibbetts MD, Reichel E, Witkin AJ. Vision loss after intravitreal ocriplasmin: correlation of spectral-domain optical coherence tomography and electroretinography. *JAMA Ophthalmol*. 2014;132:487-490.
- Fahim AT, Khan NW, Johnson MW. Acute panretinal structural and functional abnormalities after intravitreal ocriplasmin injection. *JAMA Ophthalmol*. 2014;132:484-486.
- Singh RP, Li A, Bedi R, et al. Anatomical and visual outcomes following ocriplasmin treatment for symptomatic vitreomacular traction syndrome. *Br J Ophthalmol*. 2014;98:356-360.